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Simulated microgravity upregulates an endothelial vasoconstrictor prostaglandin

DEEPALI S. SANGHA, SUKGU HAN, AND RALPH E. PURDY
Department of Pharmacology, College of Medicine, University of California, Irvine, California 92697-4625

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Sangha, Deepali S., Sukgu Han, and Ralph E. Purdy. Simulated microgravity upregulates an endothelial vasoconstrictor prostaglandin. *J Appl Physiol* 91: 789–796, 2001.—Endothelial nitric oxide contributes to the vascular hyporesponsiveness to norepinephrine (NE) observed in carotid arteries from rats exposed to simulated microgravity. The goal of the present study was to determine whether a cyclooxygenase product of arachidonic acid also influences vascular responsiveness in this setting. Microgravity was simulated in rats by hindlimb unweighting (HU). After 20 days of HU, carotid arteries were isolated from control and HU-treated rats, and vascular rings were mounted in tissue baths for the measurement of isometric contraction. Two cyclooxygenase inhibitors, indomethacin and ibuprofen, and the selective thromboxane A_2 prostanoid-receptor antagonist, SQ-29548, had no effect on the contraction to NE in control vessels but markedly reduced contraction to NE in HU vessels. When the endothelium was removed, indomethacin no longer had any effect on the NE-induced contraction in HU vessels. In endothelium-intact vessels in the presence of indomethacin, the addition of the nitric oxide synthase inhibitor, N^G-L-nitro-arginine methyl ester, to the medium bathing HU vessels increased the contraction to NE to the level of that of the control vessels. These results indicate that HU treatment induced two endothelial changes in carotid artery that opposed each other. Nitric oxide activity was increased and was responsible for the vascular hyporesponsiveness to NE. The activity of a vasoconstrictor prostaglandin was also increased, and attenuated the vasodilating effect of nitric oxide.

cyclooxygenase; endothelium; hindlimb unweighting; nitric oxide; vasoconstriction

HUMANS EXPOSED TO MICROGRAVITY undergo deconditioning of the cardiovascular system. Moreover, the severity of problems associated with reentry into the gravitational field increases with the duration of exposure to microgravity (51). A similar cardiovascular deconditioning is also observed with prolonged 6° head-down-tilt bed rest (HDTB; Ref. 51). Exposure to either microgravity or HDTB causes an immediate cephalad shift of body fluids and a subsequent sustained reduction in plasma volume and blood volume (51). Fluid redistribution to the upper half of the body results in a characteristic facial puffiness. This fluid redistribution is proposed to be the triggering event for subsequent adaptations of the cardiovascular system to microgravity or prolonged HDTB (51). On resumption of an upright posture after either HDTB or reentry into the gravitational field, subjects experience a number of adverse cardiovascular consequences, including resting tachycardia, decreased exercise capacity, and orthostatic intolerance characterized by either presyncopal symptoms or syncope. It has been hypothesized that these adverse effects may be related to hypovolemia, an attenuated reflex activation of sympathetic nervous system activity, reduced total peripheral resistance, and changes in peripheral vascular reactivity (2, 3, 15, 33, 38).

Hindlimb unweighting (HU) in rodents is used to simulate microgravity-induced cardiovascular deconditioning in humans because it elicits many of the cardiovascular alterations seen in humans. These include an initial cephalad fluid shift (20, 31, 52) and subsequent hypovolemia (35, 45), tachycardia (32), and reduced exercise capacity (14, 37).

A number of in vitro studies have investigated the effect of HU on vascular function. Delp and co-workers (13) found that the contraction of isolated rat aorta to various vasoconstrictor agents was reduced after 14-day HU. Using arterial tissues from 20-day HU-treated rats, Purdy and co-workers (38) also reported a significant decrease in the contraction of the carotid and femoral artery and the abdominal aorta to either norepinephrine or 68 mM K^+. Sangha and co-workers (41) showed that this vascular hyporesponsiveness to norepinephrine in the carotid and femoral arteries, but not the abdominal aortas, was in part due to upregulation of nitric oxide-dependent vasodilator mechanisms. In the carotid artery these vasodilator mechanisms were eliminated by removal of the endothelium (42).

The effect of endothelium removal described above prompted our hypothesis that the hyporesponsiveness to norepinephrine in the HU carotid artery was associated with changes in endothelial nitric oxide function (42). Thus, in the present study, we wanted to explore whether changes in the endothelial cyclooxygenase (COX)-dependent pathway might also occur in re-

Address for reprint requests and other correspondence: R. E. Purdy, Dept. of Pharmacology, College of Medicine, Univ. of California, Irvine, CA 92697-4625 (E-mail: repurdy@uci.edu).

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sponse to HU. Endothelial cells lining the lumen of all blood vessels modulate vascular tone through the release of vasoactive agents such as nitric oxide, eicosanoids, prostacyclin, and thromboxane (50). Nitric oxide and prostacyclin are potent vasorelaxants and inhibitors of platelet aggregation that counterbalance the vasoconstrictor and platelet-aggregating properties of thromboxane (7). Nitric oxide is formed from L-arginine by nitric oxide synthase, and the constitutive form of this enzyme predominates in endothelial cells (28). Eicosanoid synthesis from arachidonic acid is regulated by the expression and activity of COX, an enzyme also reported to be expressed in endothelial cells (4). Prostacyclin, a downstream product of COX, has been shown to serve as an endothelium-derived relaxing factor in certain blood vessels (34). Alternatively, other endothelium-derived COX metabolites are suggested to oppose vasodilation. For example, Cirino et al. (4) proposed that the release of thromboxane A_2 from the endothelial cells may attenuate the profound vasodilatation observed during shock states. Iwama and co-workers (23) showed, in the aortas from spontaneously hypertensive rats, the presence of a COX-dependent vasoconstrictor metabolite that attenuated endothelium-dependent relaxations. The aim of the present study was to determine the role of endothelium-derived, COX-dependent metabolites of arachidonic acid in the reduced contractile response to norepinephrine in carotid artery rings from 20-day HU-treated rats.

METHODS

Animal procedures were approved by the Institutional Animal Care and Use Committee of the University of California, Irvine. Male Wistar rats weighing 250–300 g were obtained from Simonsen Laboratories (Gilroy, CA) and housed in a temperature-controlled room (22°C) with a 12:12-h light-dark cycle. Water and rat chow were provided ad libitum. Animals were randomly assigned to control or HU groups. HU was achieved by using a tail harness to elevate the hindlimbs of the animals above the floor of the cage by a modification (38) of the method of Thomason et al. (48). Briefly, the tail was cleaned and a coat of tincture of benzoin was applied. It was then air dried until tacky. Adhesive strips (Fas-Trac of California, Van Nuys, CA) that were the width of the tail were looped through a swivel harness and pressed along the sides of the tail to form a tubular casing around the tail. Thereafter, the tail was wrapped with Elastoplast bandage (Beiersdorf, Norwalk, CT) followed by a thin layer of plaster cast material (Sammons Preston, Bolingbrook, IL). The rat was suspended by the swivel harness from a hook at the top center of the suspension cage, allowing free 360° rotation. The height of the hook was adjusted such that the front limbs were in contact with the floor and the hindlimbs, when extended, were elevated ~0.5 cm above the floor, tilting the body of the rat to an angle of 35° with the floor of the cage. The animals were HU for 20 days, after which the HU and the paired control rats were used in the following in vitro experiments.

Carotid artery ring preparation. HU and control rats were euthanized by exposure to 100% CO_2 for 60 s to induce deep anesthesia (19). The chest was opened and the heart removed. The carotid arteries were collected and placed in warm oxygenated Krebs bicarbonate solution. The tissues were cleaned under a dissecting microscope to remove extraneous fatty and connective tissue and then cut into 3-mm rings. In protocols examining responses in the absence of vascular endothelium, the endothelium was removed by using two strands of 32-gauge stainless steel wire tightly twisted to form a single wire bundle of 4-cm length. This bundle fitted easily into the lumen of the carotid artery rings and was used to remove the endothelium by gently rolling these tissues back and forth on moistened filter paper. Success of the endothelium removal in both control and HU vessels was confirmed by the loss of acetylcholine-mediated relaxation of phenylephrine-precontracted vessels.

In vitro isometric contraction experiments. Carotid artery rings were mounted in tissue baths for the measurement of isometric contraction, and they were stretched to previously determined optimal resting forces (38). In another study from the present laboratory (26), it was found that HU treatment reduced the contractile response to norepinephrine but not to serotonin. This implies that the effect of HU treatment is not mediated by possible vascular smooth muscle remodeling or vessel atrophy, and it validates our use of previously determined optimal resting forces: control carotid artery, 1.5 g; HU carotid artery, 1 g (38). Vessel rings were equilibrated for 30 min in 37°C,95% O_2-5% CO_2 gassed Krebs bicarbonate solution of the following composition (in mM): 119.2 NaCl, 4.9 KCl, 1.3 $CaCl_2$, 1.2 $MgSO_4$, 25 $NaHCO_3$, 11.1 glucose, 0.114 ascorbic acid, and 0.03 tetrasodium ethylenediaminetetraacetate. Subsequently, the artery rings were contracted with Krebs solution containing 100 mM K^+ prepared by equimolar replacement of Na^+. When the tissues had reached steady-state contraction, they were washed twice with normal Krebs solution and allowed to relax to resting levels. Contractions with 100 mM K^+ were repeated 20–30 min later. Preliminary experiments revealed that all tissues yielded uniform magnitudes of contraction to the second and all subsequent exposures to 100 mM K^+. Thirty to forty-five minutes after the tissues recovered from the second exposure to 100 mM K^+, norepinephrine concentration-response curves (CRCs) were obtained by the cumulative addition of norepinephrine in 0.5-log increments. Indomethacin, ibuprofen, the thromboxane A_2 prostanoid-receptor antagonist, SQ-29548, or vehicle were added to the tissue baths 30 min before the norepinephrine CRC. The following agents were also added to the bathing medium 30 min before norepinephrine CRCs were obtained: 30 μM cocaine and 30 μM deoxycorticosterone acetate to block neuronal (17) and extraneuronal (25, 27) catecholamine uptake, respectively, and 1 μM propranolol to block β-adrenoceptors (1).

Isometric contractions were recorded using either Grass FT03C (Grass Instruments, Quincy, MA) or Fort 10 Load Cell force transducers (World Precision Instruments, Sarasota, FL) connected to MacLab Electronic Data Acquisition Systems (Castle Hill, Australia). All agents were added to the bathing medium in volumes of 100 μl or less. Norepinephrine, indomethacin, and ibuprofen solutions were prepared fresh each day, whereas stock solutions of SQ 29548, cocaine, deoxycorticosterone acetate, propranolol, and N^G-nitro-L-arginine methyl ester (L-NAME) were prepared weekly and maintained at 4°C. Deoxycorticosterone acetate was dissolved in 50% ethanol, indomethacin was dissolved in 8% Na_2CO_3, and all other drugs were dissolved in double-distilled water.

In the legends of Figs. 1–6, the number of animals used for each CRC is indicated by *n*. Four rings of carotid artery were obtained from each animal. Thus two control and two treated rings from both control and HU rats were used in each

experiment, and each pair was averaged to generate one n value. Contractile responses to drugs are presented as absolute values in grams of force development. CRCs were compared by repeated measures, one-way analysis of variance using the SuperANOVA software (Abacus Concepts, Berkeley, CA), and differences between individual points on different CRCs were analyzed by a post hoc Student-Newman-Keuls t-test. $P < 0.05$ was used as the criterion for significance.

RESULTS

An earlier study from the present laboratory indicated that HU treatment resulted in hyporesponsiveness to norepinephrine in arterial tissues (38). Removal of the endothelium restored the observed hyporesponsiveness to norepinephrine toward control in the carotid artery rings from HU-treated rats (41). The purpose of the present study was to assess the possible contribution of endothelium-derived, COX-dependent arachidonic acid metabolites to the vascular hyporesponsiveness to norepinephrine in the carotid artery rings from HU-treated rats. The first approach was to expose the carotid artery rings from HU and control rats to 10 μM indomethacin, a COX inhibitor, before norepinephrine CRCs were obtained. Addition of either indomethacin or ibuprofen (see below) had no effect on resting force. As observed in our laboratory's earlier studies (38), HU treatment resulted in a significantly reduced response to norepinephrine in the carotid artery (Fig. 1). Indomethacin did not affect the response to norepinephrine in control arteries, except at the highest norepinephrine concentration used (300 μM). In this case, indomethacin increased the contraction. In contrast, indomethacin caused a further depression in the contractile response to norepinephrine in the HU-treated carotid artery rings. In six of the eight experiments shown in Fig. 1, HU tissues contracted weakly to norepinephrine in the presence of indomethacin. However, norepinephrine elicited no response in two experiments, resulting in a nonsignificant effect of norepinephrine in these tissues. To confirm these results, the effects of a structurally different COX inhibitor, 100 μM ibuprofen, were assessed. Ibuprofen had no effect on control carotid artery rings but significantly depressed the contractile response to norepinephrine in the carotid artery rings from HU-treated rats (Fig. 2).

Taken together, these results indicated that HU treatment upregulated the activity of a COX-dependent vasoconstrictor substance. PGH_2 and thromboxane A_2 are known vasoconstrictor agents that exert their effects through the thromboxane A_2 prostanoid receptor (6). Thus experiments were carried out to assess the consequences of selective thromboxane A_2 prostanoid-receptor blockade using SQ-29548 (36). As shown in Fig. 3, 0.1 μM SQ-29548 had no effect on control carotid artery rings but further depressed the contraction to norepinephrine in HU vessel rings. The similar effects of COX and thromboxane A_2 prostanoid-receptor inhibition suggested that the contractile response to norepinephrine in HU carotid artery rings

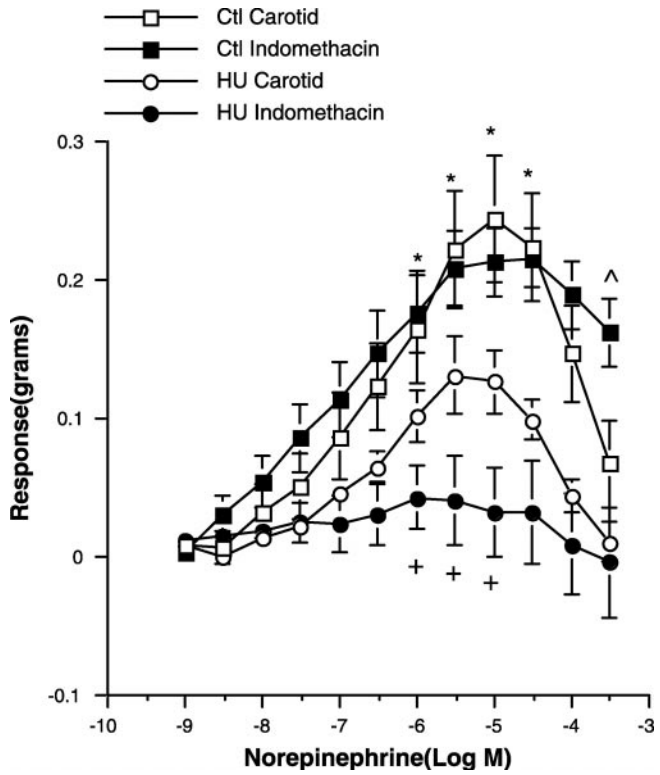

Fig. 1. Concentration-response curves for the contractile effects of norepinephrine in control (Ctl) and hindlimb unweighted (HU) carotid artery rings with and without 10 μM indomethacin. Values are means ± SE; $n = 5$ animals per treatment group. *HU different from control, $P < 0.05$. $^+$HU different from HU in the presence of indomethacin, $P < 0.05$. ^ Control different from control in the presence of indomethacin, $P < 0.05$.

was mediated, in part, by a product of the COX pathway that acted on the thromboxane A_2 prostanoid receptor.

To determine whether the COX-dependent vasoconstrictor metabolite was derived from the endothelium, norepinephrine CRCs were obtained in endothelium-denuded carotid artery rings in the presence and absence of indomethacin. As shown in Fig. 4, indomethacin had no effect on the norepinephrine CRC in HU carotid artery. This result demonstrates that the COX-dependent vasoconstrictor metabolite was derived from the endothelium. In addition, indomethacin had no effect on the norepinephrine CRC in control carotid artery.

In a previous study, it was observed that endothelium removal increased the maximal contraction of HU carotid artery to norepinephrine (42). As a consequence, that contraction moved closer to the maximal norepinephrine-induced contraction in the control carotid artery. Inspection of Fig. 4 revealed a similar result. The maximal response to norepinephrine in HU was closer to that in control carotid artery in endothelium-denuded (34%; Fig. 4), compared with endothelium-intact (46%; Fig. 1), vessels. However, the CRCs in the control and HU denuded carotid arteries were clearly not superimposed. Statistical analysis found no significant differences between the two CRCs. How-

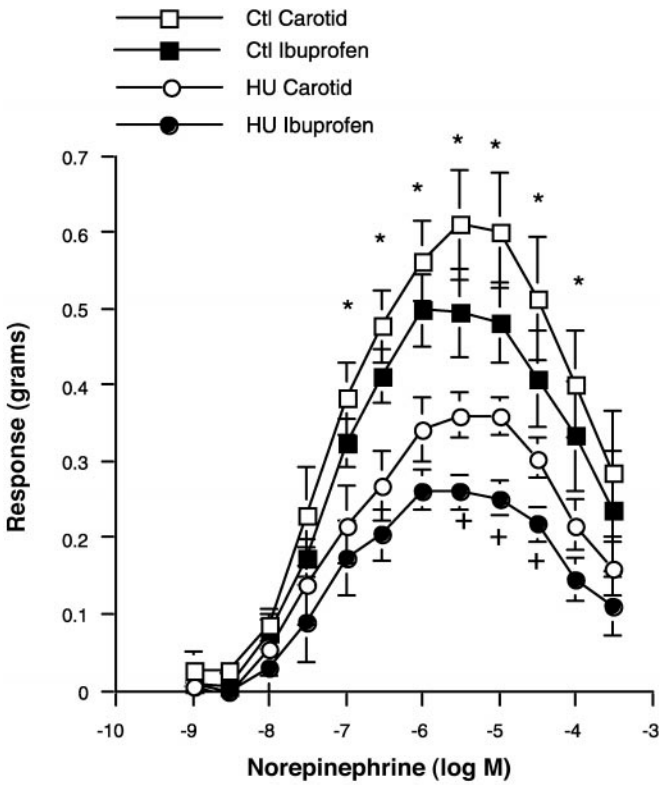

Fig. 2. Concentration-response curves for the contractile effects of norepinephrine in Ctl and HU carotid artery rings with and without 100 μM ibuprofen. Values are means ± SE; $n = 5$ animals per treatment group. *HU different from control, $P < 0.05$. $^{+}$HU different from HU in the presence of ibuprofen. $P < 0.05$.

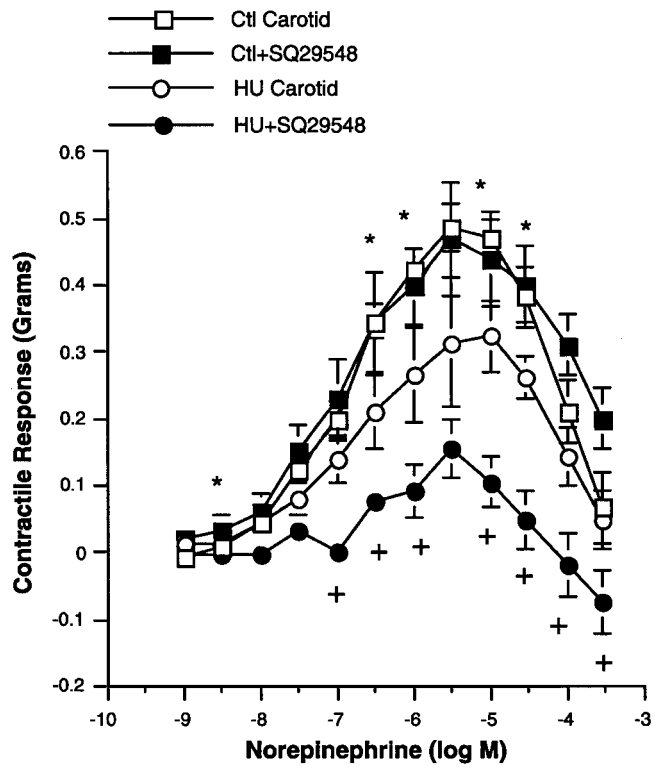

Fig. 3. Concentration-response curves for the contractile effects of norepinephrine in Ctl and HU carotid artery rings with and without 0.1 μM SQ-29548. Values are means ± SE; $n = 5$ animals per treatment group. *HU different from control, $P < 0.05$. $^{+}$HU different from HU in the presence of SQ-29548, $P < 0.05$.

ever, power calculations revealed that, for contractions to 1–10 μM norepinephrine, there was insufficient statistical power to establish a lack of significant difference between these two CRCs.

The effect of the nitric oxide synthase inhibition with 10 μM L-NAME was assessed in endothelium-intact control and HU carotid artery rings pretreated with indomethacin to eliminate the contribution of a vasoconstrictor prostaglandin. Addition of L-NAME had no effect on resting force. As shown in Fig. 5, HU treatment markedly depressed the contraction to norepinephrine. However, the contractile response of the HU carotid artery rings in the presence of L-NAME increased and the norepinephrine CRC became superimposed on that of the control tissues in the absence of L-NAME. L-NAME also markedly increased the contractile response to norepinephrine in the control tissues. Thus the HU-induced reduction in contractile response was retained in the presence of indomethacin plus L-NAME.

Additional experiments were carried out to determine the effect of L-NAME alone, i.e., in the absence of indomethacin. As shown in Fig. 6, the norepinephrine CRC in the HU carotid artery was depressed, compared with control. However, in the presence of L-NAME, the HU CRC was elevated and became superimposed on the control CRC in the absence of L-NAME. L-NAME had no significant effect in control carotid

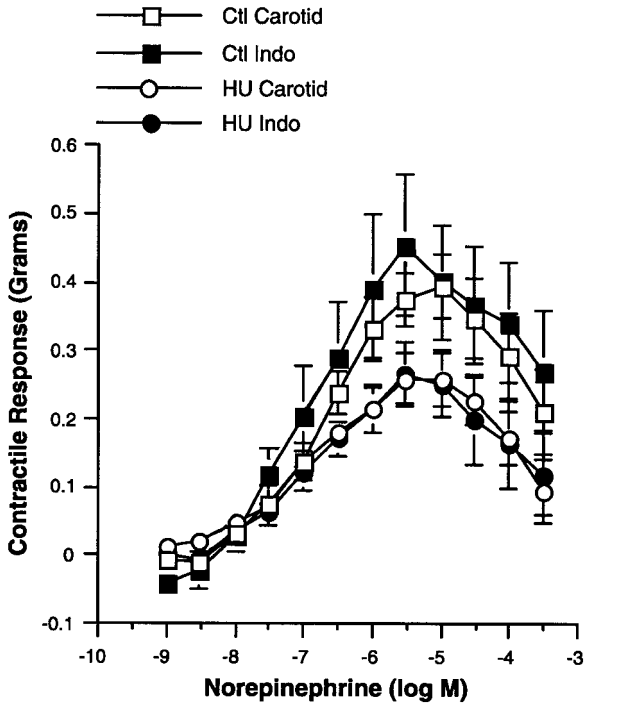

Fig. 4. Concentration-response curves for the contractile effects of norepinephrine in endothelium-denuded Ctl and HU carotid artery rings with and without 10 μM indomethacin (Indo). Values are means ± SE; $n = 8$ animals per treatment group.

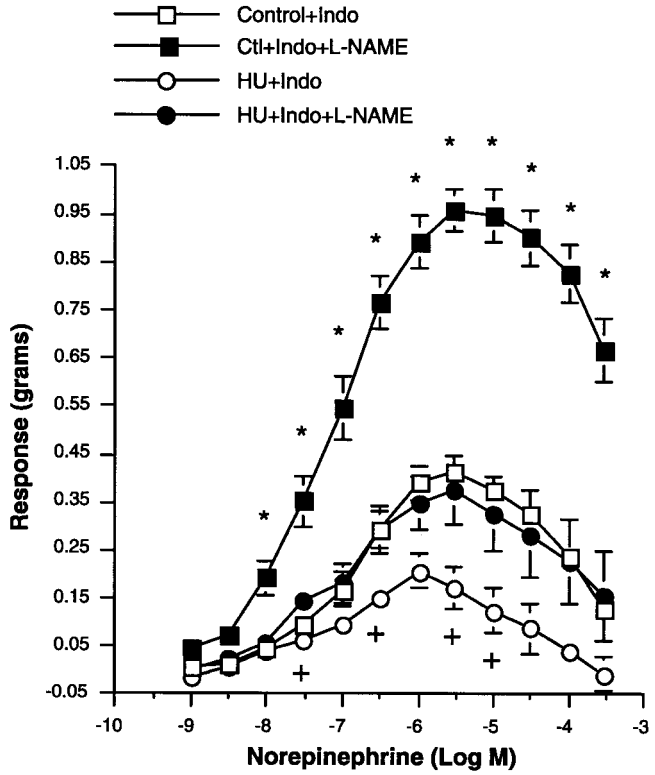

Fig. 5. Concentration-response curves for the contractile effects of norepinephrine in Ctl and HU carotid artery rings in the presence of 10 μM Indo. HU vessels are with and without the nitric oxide inhibitor N^G-nitro-L-arginine methyl ester (L-NAME; 10 μM). Values are means ± SE; $n = 5$ animals per treatment group. *HU different from control, $P < 0.05$. $^+$HU different from HU in the presence of L-NAME. $P < 0.05$.

arteries but tended to cause an increase. L-NAME treatment also increased the variability of vessel contraction, as indicated by larger standard errors of the mean.

DISCUSSION

HU treatment causes vascular hyporesponsiveness to norepinephrine in several arteries of the rat (10, 11, 13, 38), including the abdominal aorta and the femoral and carotid arteries. Results from a recent study (42) provided evidence that nitric oxide derived from the endothelium contributed to the HU-induced hyporesponsiveness of the carotid artery. Also, the present finding (Fig. 4) that endothelium removal reduced the HU effect is consistent with such a role for nitric oxide. The present study was undertaken to explore whether COX-dependent metabolites of arachidonic acid might also contribute to the effect of HU treatment.

HU treatment depressed the contractile response of the carotid artery to norepinephrine, as reported previously (38). Pretreatment with either of two different COX inhibitors caused a further depression of the contractile response to norepinephrine in HU carotid artery rings but had no effect in control artery rings. Importantly, the thromboxane A_2 prostanoid-receptor antagonist, SQ-29548, mimicked these effects of COX inhibition. Taken together, these results suggested that a COX-dependent metabolite of arachidonic acid acted on thromboxane A_2 prostanoid receptors to mediate a large component of the contractile response to norepinephrine in HU carotid artery. Finally, endothelium removal eliminated the blocking effect of indomethacin in the HU carotid artery (see Fig. 4). This demonstrated that the COX-dependent metabolite was derived from the endothelium.

Endothelium removal was shown previously (42) and in the present study (Fig. 4) to increase the contraction of HU carotid artery to norepinephrine. In our laboratory's earlier study (42), this was the first evidence to suggest that HU treatment might upregulate endothelial vasodilator function. This suggestion was strengthened by observations that HU treatment caused *1*) a 10-fold increase in the sensitivity of carotid artery to acetylcholine-induced vasodilation (42) and *2*) a significant increase in endothelial nitric oxide synthase expression in this vessel (41).

In endothelium-denuded vessels, the norepinephrine CRC in HU carotid artery approached, but did not become superimposed on, the CRC in the control vessel (Fig. 4). If HU-induced hyporesponsiveness to norepinephrine were due to an endothelium-dependent mechanism exclusively, endothelium removal should have caused the CRCs in control and HU carotid arteries to become superimposed. Because they were not superimposed, we suggest that HU, in addition to its endothelial effect, impaired vascular smooth muscle

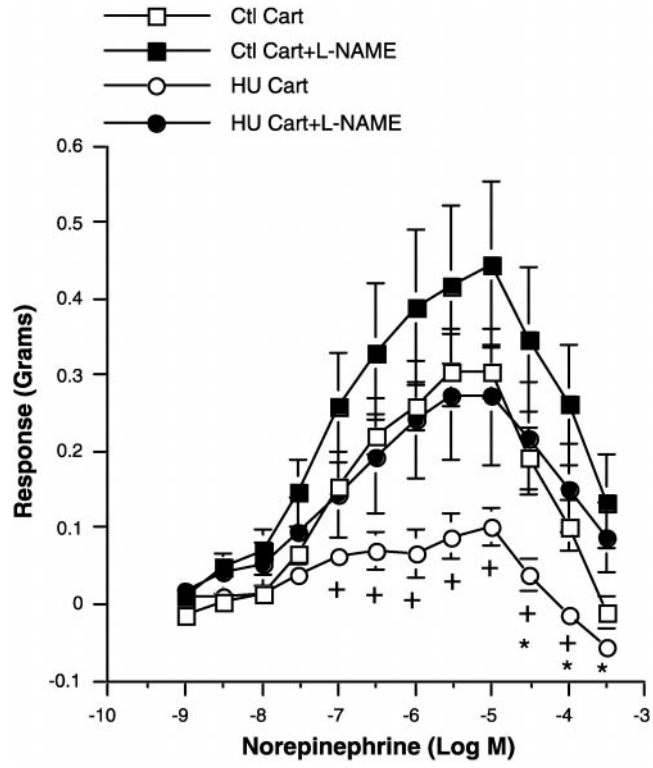

Fig. 6. Concentration-response curves for the contractile effects of norepinephrine in Ctl and HU carotid artery (Cart) rings with and without 10 μM L-NAME. Values are mean ± SE; $n = 5$ animals per treatment group. *HU different from HU+L-NAME, $P < 0.05$; $^+$HU different from control, $P < 0.05$.

function, thereby contributing to the depression of contractility in the carotid artery. Power calculations revealed that the eight experiments represented in Fig. 4 of the present study were insufficient to detect a significant HU effect in denuded carotid artery. Additional research is required to address this issue.

Experiments were carried out with indomethacin to block COX and with L-NAME to block nitric oxide synthase. It is important to note that these agents were added to the bathing medium after the artery rings were equilibrated at their optimum resting forces. Neither indomethacin nor L-NAME had any effect on resting force. This has several implications. First, we suggest that there was little or no basal synthesis and release of a vasoconstrictor prostaglandin. If there had been, this prostaglandin would have produced baseline tone and the addition of indomethacin would have caused the vessel to relax. This argument also supports the view that the indomethacin-sensitive component of the contraction to norepinephrine was not due to the spontaneous release of the vasoconstrictor prostaglandin. Rather, it appears that the production of the prostaglandin was dependent on the activation of α-adrenoceptors. It is possible that, in some presently unknown way, HU treatment coupled vasoconstrictor prostaglandin production as a second messenger to the α-adrenoceptor. Alternatively, HU treatment could simply have increased the expression of the synthesis pathway for the prostaglandin. In this case, synthesis and release could have been triggered by cellular shape changes associated with the norepinephrine-induced contraction. Further research is required to differentiate between these possibilities.

Second, the lack of effect of L-NAME on resting tone has two possible interpretations. It may indicate that there is no spontaneous release of nitric oxide. This interpretation presupposes that the carotid artery must possess myogenic tone. If true, that tone could be suppressed by spontaneously released nitric oxide. In that case, L-NAME would have blocked nitric oxide synthesis and release and unmasked the myogenic tone. If this scenario were true, the lack of tone development would suggest that there is no spontaneous release of nitric oxide. Alternatively, the carotid artery may not possess myogenic tone. In this case, blockade of nitric oxide release by L-NAME would not have an effect under any circumstances. These two possibilities cannot be distinguished by the present results.

Throughout most of the norepinephrine CRC, indomethacin had no effect on the contraction of control carotid arteries. This implies that activation of α-adrenoceptors in control arteries was not associated with prostaglandin. In contrast, indomethacin (and also ibuprofen and SQ-29548) further suppressed an already decreased contraction to norepinephrine in HU carotid arteries. This effect of indomethacin on HU carotid artery contractions was eliminated in endothelium-denuded vessels. These observations support the major conclusion of this study that HU treatment induces the expression of the synthetic machinery for a vasoconstrictor prostaglandin within the endothelium. Moreover, synthesis and release of such a prostaglandin contributed to the contraction of HU carotid artery to norepinephrine.

These results and those of our laboratory's earlier studies suggest that HU treatment upregulated both a vasodilator, nitric oxide (42), and a vasoconstrictor, prostaglandin (present study), in the endothelium of carotid artery. To explore this further, it was hypothesized that blockade of both nitric oxide synthase and COX would have an effect equivalent to endothelium removal. Namely, the norepinephrine-induced contraction in HU arteries would more closely approximate that in the control arteries in the presence of L-NAME and indomethacin. However, the results obtained were far more complex, at least with respect to control carotid arteries, than this hypothesis suggests. First, it should be noted that L-NAME increased the contraction of HU arteries to norepinephrine, both in the presence (Fig. 5) and absence (Fig. 6) of indomethacin. This is consistent with an enhanced role for nitric oxide in HU carotid artery. What was unexpected was the dramatic increase in the contraction of control carotid arteries to norepinephrine in the presence of both L-NAME and indomethacin (Fig. 5). We offer the following speculation. In Fig. 1, it can be seen that indomethacin had no effect on the contraction of control carotid artery except at the highest concentration of norepinephrine, 300 μM. The fact that indomethacin enhanced the contraction to this concentration of norepinephrine suggests the involvement of a vasodilator prostaglandin. In Fig. 6, it can be seen that the nitric oxide synthase inhibitor, L-NAME, caused a modest, nonsignificant elevation of the norepinephrine CRC in control carotid artery. Thus it is possible that control arteries possess two potential vasodilator pathways, a prostaglandin and nitric oxide. Blocking either of these pathways has little effect because they are both relatively weak and/or each can compensate for the other. However, blocking both pathways has a synergistic effect that manifests as a marked enhancement of contraction.

There are precedents in other animal models for an interaction between nitric oxide and a COX-dependent vasoconstrictor substance, both released from the endothelium. Koga et al. (29) and Iwama et al. (23) assessed the maximal acetylcholine-induced relaxation of precontracted aorta rings from spontaneously hypertensive and normotensive rats. They found that this relaxation was attenuated by both age and hypertension. This relaxation was blocked by L-NAME, indicating that it was mediated by nitric oxide. Importantly, the attenuation of this relaxation was reversed by either COX inhibition (29) or thromboxane A_2 prostanoid-receptor blockade (23). Thus both studies (23, 29) provided evidence that a COX-dependent vasoconstrictor substance had been released from the endothelium and that this substance accounted for the age and hypertension-induced attenuation of relaxation to acetylcholine.

The mechanisms responsible for the HU-mediated induction of an endothelially derived, COX-dependent

vasoconstrictor substance in carotid artery are unknown. However, there is evidence to support the hypothesis that the upregulated nitric oxide itself may have stimulated the production of the COX metabolite. A direct interaction of the nitric oxide and COX pathways has been reported for various cell types and tissues (16, 40, 47). Davidge et al. (7) observed an activation of COX by nitric oxide in cultured microvascular endothelial cells. This interaction could have occurred via intermediary pathways as follows. Under conditions of enhanced oxidative stress, nitric oxide reacts with superoxide anions to form peroxynitrite (22). This could lead to an increase in lipid peroxidation that would enhance COX activity (21). Using Western immunoblots of the thoracic aorta, Davidge et al. (8) reported an increased COX expression in older rats and suggested that a natural age-related increase in endogenous lipid peroxidation may have increased COX expression in these rats. Whether the HU-induced increase in endothelially derived nitric oxide in carotid artery was responsible for induction of the COX-dependent metabolite observed in the present study is unknown. Future experiments will be required to elucidate this issue. However, endothelial nitric oxide does not contribute to the hyporesponsiveness to norepinephrine in either abdominal aorta (13, 42) or femoral artery from HU rats (42). Moreover, in contrast to the carotid artery, COX inhibition has no effect on the contraction of these vessels to norepinephrine (26; Sangha and Purdy, unpublished observations). This lends further support to the possibility that a causal relationship exists between elevated endothelial nitric oxide activity and the induction of an endothelium-derived vasoconstrictor prostaglandin.

The mechanisms underlying the HU-induced upregulation of endothelial nitric oxide function (41, 42) in carotid artery are unknown. However, endothelial nitric oxide vasodilator activity is regulated by changes in blood flow and associated shear stress (44). We speculate that HU increased shear stress in the carotid artery by causing a cephalad fluid shift (20, 31, 52). In turn, this could have increased endothelial nitric oxide function.

An increase in endothelial nitric oxide function is not a universal feature of blood vessels from the HU rat. Jasperse et al. (24) found that endothelial nitric oxide synthase protein expression was reduced in rat soleus muscle feed arteries after 2 wk of HU. In addition, these authors and Delp et al. (12) found that the vasodilation of soleus feed arteries to acetylcholine was reduced. These observations may also reflect HU-induced changes in shear stress. Delp et al. found that shear stress was reduced in soleus feed arteries after 2 wk of HU.

Geary et al. (18) obtained functional evidence, in rat cerebral artery, that HU reduced nitric oxide activity. Because this was responsible, in part, for an increase in myogenic tone in this vessel, these authors suggested that the reduced nitric oxide activity was an appropriate adaptation. They suggested that elevated cerebrovascular myogenic tone and consequent vasoconstriction could act to protect the brain from overperfusion. In view of this argument, the hyporesponsiveness of the carotid artery seems inappropriate with respect to proper maintenance of brain blood flow. We suggest that the main effect of carotid artery hyporesponsiveness is to contribute to orthostatic hypotension, a known consequence of both simulated (30) and real (51) microgravity.

In summary, the present study confirms our laboratory's earlier observation (42) that HU treatment increases endothelium-dependent, nitric oxide-mediated vascular mechanisms in the carotid artery. In addition, we have uncovered an endothelium-dependent vasoconstrictor component of the response of HU treated carotid arteries to norepinephrine. This component appears to compensate partially for the HU-induced vascular hyporesponsiveness in this blood vessel. It is unknown whether exposure of humans in microgravity produces similar endothelium-dependent vasodilator and vasoconstrictor changes. The present finding of an apparent COX-dependent vasoconstrictor component raises the caution that the use of COX inhibitors by astronauts may have the potential to exacerbate postural intolerance by further depressing the ability of arteries to constrict to the endogenous neurotransmitter, norepinephrine.

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REFERENCES

1. **Besse JC and Furchgott RF.** Dissociation constants and relative efficacies of agonists acting on alpha-adrenergic receptors in rabbit aorta. *J Pharmacol Exp Ther* 197: 66–78, 1976.
2. **Billman GE, Dickey DT, Sandler H, and Stone HL.** Effects of horizontal body casting on the baroreflex control of the heart. *J Appl Physiol* 52: 1552–1556, 1982.
3. **Buckey JC Jr, Lane LD, Levine BD, Watenpaugh DE, Wright SJ, Moore WE, Gaffney FA, and Blomqvist CG.** Orthostatic intolerance after spaceflight. *J Appl Physiol* 81: 7–18, 1996.
4. **Cirino G, Sorrentino R, Cicala C, Sorrentino L, and Pinto A.** Indomethacin and thromboxane A_2/prostaglandin H_2 antagonist SQ 29548 impair in vitro contractions of aortic rings of ex vivo treated lipopolysaccharide rats. *J Lipid Mediat* 13: 177–187, 1996.
5. **Cohen RA.** Dysfunction of vascular endothelium in diabetes mellitus. *Circulation* 87: 67–76, 1993.
6. **Coleman RA, Smith WL, and Narumiya S.** International Union of Pharmacology classification of prostanoid receptors: properties, distribution, and structure of the receptors and their subtypes. *Pharmacol Rev* 46: 205–229, 1994.
7. **Davidge ST, Baker PN, McLaughlin MK, and Roberts JM.** Nitric oxide produced by endothelial cells increases production of eicosanoids through activation of prostaglandin H synthase. *Circ Res* 77: 274–283, 1995.
8. **Davidge ST, Hubel CA, and McLaughlin MK.** Impairment of vascular function is associated with an age-related increase in lipid peroxidation in rats. *Am J Physiol Regulatory Integrative Comp Physiol* 271: R1625–R1631, 1996.
9. **Deiderich D, Yang Z, Buhler FR, and Luscher TF.** Impaired endothelium-dependent relaxations in hypertensive resistance arteries involves cyclooxygenase pathways. *Am J Physiol Heart Circ Physiol* 258: H445–H451, 1990.

10. **Delp MD.** Myogenic and vasoconstrictor responsiveness of skeletal muscle arterioles is diminished by hindlimb unloading. *J Appl Physiol* 86: 1178–1184, 1999.
11. **Delp MD, Brown M, Laughlin MH, and Hasser EM.** Rat aortic vasoreactivity is altered by old age and hindlimb unloading. *J Appl Physiol* 78: 2079–2086, 1995.
12. **Delp MD, Colleran P, Wilkerson K, McCurdy M, and Muller-Delp J.** Structural and functional remodeling of skeletal muscle microvasculature is induced by simulated microgravity. *Am J Physiol Heart Circ Physiol* 278: H1866–H1873, 2000.
13. **Delp MD, Holder-Binkley T, Laughlin MH, and Hasser EM.** Vasoconstrictor properties of rat aorta are diminished by hindlimb unweighting. *J Appl Physiol* 75: 2620–2628, 1993.
14. **Desplanches D, Mayet MH, Sempore B, and Flandrois R.** Structural and functional responses to prolonged hindlimb suspension in rat muscle. *J Appl Physiol* 63: 558–563, 1987.
15. **Dickey DT, Billman GE, Teoh K, Sandler H, and Stone HL.** The effects of horizontal body casting on blood volume, drug responsiveness, and +GZ tolerance in the rhesus monkey. *Aviat Space Environ Med* 53: 142–146, 1982.
16. **Franchi AM, Chaud M, Rettori V, Suburo A, McCann SM, and Gimeno M.** Role of nitric oxide in eicosanoid synthesis and uterine motility in estrogen-treated rat uteri. *Proc Natl Acad Sci USA* 91: 539–543, 1994.
17. **Furchgott RF** The classification of adrenoceptors (adrenergic receptors). An evaluation from the standpoint of receptor theory. In: *Handbuch der experimentellen Pharmakologie*. *Catecholamines*, edited by Blaschko H and Muscholl E. New York: Springer-Verlag, 1972, vol. 33, p. 283–335.
18. **Geary G, Krause D, Purdy R, and Duckles S.** Simulated microgravity increases myogenic tone in rat cerebral arteries. *J Appl Physiol* 85: 1615–1621, 1998.
19. **Glen JB and Scott WN.** Carbon dioxide euthanasia of cats. *Br Vet J* 129: 471–479, 1973.
20. **Hargens AR, Steakai J, Johansson C, and Tipton CM.** Tissue fluid shift, forelimb loading, and tail tension in tail suspended rats. *Physiologist* 27, *Suppl*: S37–S38, 1984.
21. **Hemler ME and Lands WEM.** Evidence for a peroxide-initiated free radical mechanism of prostaglandin biosynthesis. *J Biol Chem* 255: 6253–6261, 1980.
22. **Huie RE and Padmaja S.** The reaction of NO with superoxide. *Free Radic Res Commun* 18: 195–199, 1993.
23. **Iwama Y, Kato T, Muramatsu M, Asano H, Shimizu K, Toki Y, Miyazaki Y, Okumura K, Hashimoto H, Ito T, and Satake T.** Correlation with blood pressure of the acetylcholine-induced endothelium-derived contracting factor in the rat aorta. *Hypertension* 19: 326–332, 1992.
24. **Jasperse JL, Woodman C, Price E, Hasser E, and Laughlin M.** Hindlimb unweighting decreases ecNOS gene expression and endothelium-dependent dilation in rat soleus feed arteries. *J Appl Physiol* 87: 1476–1482, 1999.
25. **Johnson SM and de la Lande IS.** Action of deoxycorticosterone acetate on the central artery of the rabbit ear. *Blood Vessels* 15: 241–246, 1978.
26. **Kahwaji CI, Sangha DS, Sheibani S, Ashori M, Nguyen H, Kim Y, and Purdy RE.** Simulated microgravity-induced vascular hyporesponsiveness: role of signal transduction. *Proc West Pharmacol Soc* 42: 9–12, 1999.
27. **Kalsner S.** Steroid potentiation of responses to sympathomimetic amines in aortic strips. *Br J Pharmacol* 36: 582–593, 1969.
28. **Knowles RG and Moncada S.** Nitric oxide synthases in mammals. *Biochem J* 298: 249–258, 1994.
29. **Koga T, Takata Y, Kobayashi K, Takishita S, Yamashita Y, and Fujishima M.** Age and hypertension promote endothelium-dependent contractions to acetylcholine in the aorta of the rat. *Hypertension* 14: 542–548, 1989.
30. **Martel E, Champeroux P, Lacolley P, Richard S, Safar M, and Cuche JL.** Central hypervolemia in the conscious rat: a model of cardiovascular deconditioning. *J Appl Physiol* 80: 1390–1396, 1996.
31. **Maurel D, Ixart G, Barbanel G, Meknouche M, and Assenmacher I.** Effects of acute tilt from orthostatic to head-down antiorthostatic restraint and of sustained restraint on the intracerebroventricular pressure in rats. *Brain Res* 736: 165–173, 1996.
32. **McDonald KS, Delp MD, and Fitts RH.** Effect of hindlimb unweighting on tissue blood flow in the rat. *J Appl Physiol* 72: 2210–2218, 1992.
33. **Moffitt JA, Foley CM, Schadt JC, Laughlin MH, and Hasser EM.** Attenuated baroreflex control of sympathetic nerve activity after cardiovascular deconditioning in rats. *Am J Physiol Regulatory Integrative Comp Physiol* 274: R1397–R1405, 1998.
34. **Moncada S, Herman AG, Higgs EA, and Vane JR.** Differential formation of prostacyclin (PGX or PGI_2) by layers of arterial wall: an explanation for the anti-thrombotic properties of vascular endothelium. *Thromb Res* 11: 323–344, 1977.
35. **Musacchia XJ, Deavers DR, and Meininger GA.** Fluid/electrolyte balance and cardiovascular responses: head-down tilted rats. *Physiologist* 33, *Suppl*: S47, 1990.
36. **Ogletree ML, Harris DN, Greenberg R, Haslanger MF, and Nakane M.** Pharmacological actions of SQ29548, a novel selective thromboxane antagonist. *J Pharmacol Exp Ther* 234: 435–441, 1985.
37. **Overton JM, Woodman CR, and Tipton CM.** Effect of hindlimb suspension on $\dot{V}o_{2\,max}$ and regional blood flow responses to exercise. *J Appl Physiol* 66: 653–659, 1989.
38. **Purdy RE, Duckles SP, Krause DN, Rubera KM, and Sara D.** Effect of simulated microgravity on vascular contractility. *J Appl Physiol* 85: 1307–1315. 1998.
39. **Rathaus M, Greenfeld Z, Podjarny E, Brezis M, and Bernheim J.** Sodium loading and renal prostaglandins in old rats. *Prostaglandins Leukot Essent Fatty Acids* 59: 815–819, 1993.
40. **Salvemini D, Misko TP, Masferrer JL, Seibert K, Currie MG, and Needleman P.** Nitric oxide activates cyclooxygenase enzymes. *Proc Natl Acad Sci USA* 90: 7240–7244, 1993.
41. **Sangha DS, Vaziri ND, Ding Y Y, Han S, Alem N, Chen YA, and Purdy RE.** Simulated microgravity impairs vascular contractility: role of nitric oxide-dependent vasodilator mechanisms. *Proc West Pharmacol Soc* 42: 5–7, 1999.
42. **Sangha DS, Vaziri ND, Ding Y, and Purdy RE.** Vascular hyporesponsiveness in simulated microgravity: role of nitric oxide dependent mechanisms. *J Appl Physiol* 88: 507–517, 2000.
43. **Sato I, Kaji K, Morita I, Nagao M, and Murota S.** Augmentation of endothelin-1, prostacyclin, and thromboxane A_2 secretion associated with in vitro aging in cultured human umbilical vein endothelial cells. *Mech Aging Dev* 71: 73–84, 1993.
44. **Schini-Kerth VB and Vanhoutte PM.** Nitric oxide synthases in vascular cells. *Exp Physiol* 80: 885–905, 1995.
45. **Shellock FG, Swan HJC, and Rubin SA.** Early central venous pressure changes in the rat during two different levels of head-down suspension. *Aviat Space Environ Med* 56: 791–795, 1985.
46. **Somlyo AP and Somlyo AV.** Vascular smooth muscle: I. Normal structure, pathology, biochemistry, and biophysics. *Pharmacol Rev* 20: 197–272, 1968.
47. **Stadler J, Harbrecht BG, Di Silvio M, Curran RD, Jordan ML, Simmons RL, and Billiar TR.** Endogenous nitric oxide inhibits the synthesis of cyclooxygenase products and interleukin-6 by rat Kupffer cells. *J Leukoc Biol* 53: 165–172, 1993.
48. **Thomason DB, Herrick RE, Surdyka D, and Baldwin KM.** Time course of soleus muscle myosin expression during hindlimb suspension and recovery. *J Appl Physiol* 63: 130–137, 1987.
49. **Todd MK, Goldfarb AH, Kaufmann RD, and Burleson C.** Combined effects of age and exercise on thromboxane B_2 and platelet activation. *J Appl Physiol* 76: 1548–1552, 1994.
50. **Vane JR, Anffard EE, and Blotting RM.** Regulatory functions of the vascular endothelium. *N Engl J Med* 323: 27–36, 1990.
51. **Watenpaugh DE and Hargens AR.** The cardiovascular system in microgravity. In: *Handbook of Physiology. Environmental Physiology*. Bethesda, MD: Am. Physiol. Soc., 1996, sect. 4, vol. I, pt. 3, chapt. 29, p 631–674.
52. **Wilkerson MK, Muller-Delp J, Colleran PN, and Delp MD.** Effects of hindlimb unloading on rat cerebral, splenic, and mesenteric resistance artery morphology. *J Appl Physiol* 87: 2115–2121, 1999.
53. **Woodman CR, Sebastian LA, and Tipton CM.** Influence of simulated microgravity on cardiac output and blood flow distribution during exercise. *J Appl Physiol* 79: 1762–1768, 1995.